

Fig. 1. Schematic representation of the steps involved in the isolation of retinoid-regulated genes using the differential display technique. The cloned products isolated in step 6 can then be used for sequencing, Northern blotting or screening of cDNA libraries. P1, P2 and P3 correspond to fragments from RA induced mRNAs. P4 represents a PCR product from an mRNA which is down-regulated.

FIG. 1

FIG. 2

A



C

MetGlyLeuTyrThrLeuMetValThrPheLeuCysThrIleValLeuProValLeuLeu 20
PheLeuAlaAlaValLysLeuTrpGluMetLeuMetIleArgArgValAspProAsnCys 40
ArgSerProLeuProProGlyThrMetGlyLeuProPheIleGlyGluThrLeuGlnLeu 60
IleLeuGlnArgArgLysPheLeuArgMetLysArgGlnLysTyrGlyCysIleTyrLys 80
ThrHisLeuPheGlyAsnProThrValArgValMetGlyAlaAspAsnValArgGlnIle 100
LeuLeuGlyGluHisLysLeuValSerValGlnTrpProAlaSerValArgThrIleLeu 120
GlySerAspThrLeuSerAsnValHisGlyValGlnHisLysAsnLysLysLysAlaIle 140
MetArgAlaPheSerArgAspAlaLeuGluHisTyrIleProValIleGlnGlnGluVal 160
LysSerAlaIleGlnGluTrpLeuGlnLysAspSerCysValLeuValTyrProGluMet 180
LysLysLeuMetPheArgIleAlaMetArgIleLeuLeuGlyPheGluProGluGlnIle 200
LysThrAspGluGlnGluLeuValGluAlaPheGluGluMetIleLysAsnLeuPheSer 220
LeuProIleAspValProPheSerGlyLeuTyrArgGlyLeuArgAlaArgAsnPheIle 240
HisSerLysIleGluGluAsnIleArgLysLysIleGlnAspAspAspAsnGluAsnGlu 260
GlnLysTyrLysAspAlaLeuGlnLeuLeuIleGluAsnSerArgArgSerAspGluPro 280
PheSerLeuGlnAlaMetLysGluAlaAlaThrGluLeuLeuPheGlyGlyHisGluThr 300
ThrAlaSerThrAlaThrSerLeuValMetPheLeuGlyLeuAsnThrGluValValGln 320
LysValArgGluGluValGlnGluLysValGluMetGlyMetTyrThrProGlyLysGly 340
LeuSerMetGluLeuLeuAspGlnLeuLysTyrThrGlyCysValIleLysGluThrLeu 360
ArgIleAsnProProValProGlyGlyPheArgValAlaLeuLysThrPheGluLeuAsn 380
GlyTyrGlnIleProLysGlyTrpAsnValIleTyrSerIleCysAspThrHisAspVal 400
AlaAspValPheProAsnLysGluGluPheGlnProGluArgPheMetSerLysGlyLeu 420
GluAspGlySerArgPheAsnTyrIleProPheGlyGlyGlySerArgMetCysValGly 440
LysGluPheAlaLysValLeuLeuLysIlePheLeuValGluLeuThrGlnHisCysAsn 460
TrpIleLeuSerAsnGlyProProThrMetLysThrGlyProThrIleTyrProValAsp 480
AsnLeuProThrLysPheThrSerTyrValArgAsn 492

B

→
TCCAGTGGACAATCTCCCTACCAATTCACCTAGTTATGTCAGAAATTA 50
GCCTAAACCCGAGCCTTTGTACATATGTTTTATTTTAGATGAACCTGGA 100
TGTATTGGATATTTTCTAATTTGTTTATATAAGCAGATGTTATATATAAG 150
TCTATGCGAAGGAGCGAAACGAGGCACTACTTTCTCATGGATCACTGT 200
AATGCTACAGAGTGTCTGTGATGTTATATTTATAATGTAAGTTGTGTATAT 250
AGCTTTTGTACTGTATGCAACTTATTTAACTCGCTCTTATCTCATGGGT 300
TTTATTTAATAAAACATGTTCTTACAAAAA 337
←

D

	-8	-4	0	4	8
P450RAI	PFGGGSRMVCGKEFAKVLK				
ATCYTP450	****P*L*P*Y*L*R*A*S				
RATCYP4A1	**S**A*N*I**Q**MSEM*				
RABCYP4A5	**S**A*N*I**Q**MNE**				
CYP4503A12	***T*P*N*I**MR**IMNM*				
hCYTFAOH	**S****N*I**Q**MNE**				

005668482 092500

005250" 23489960

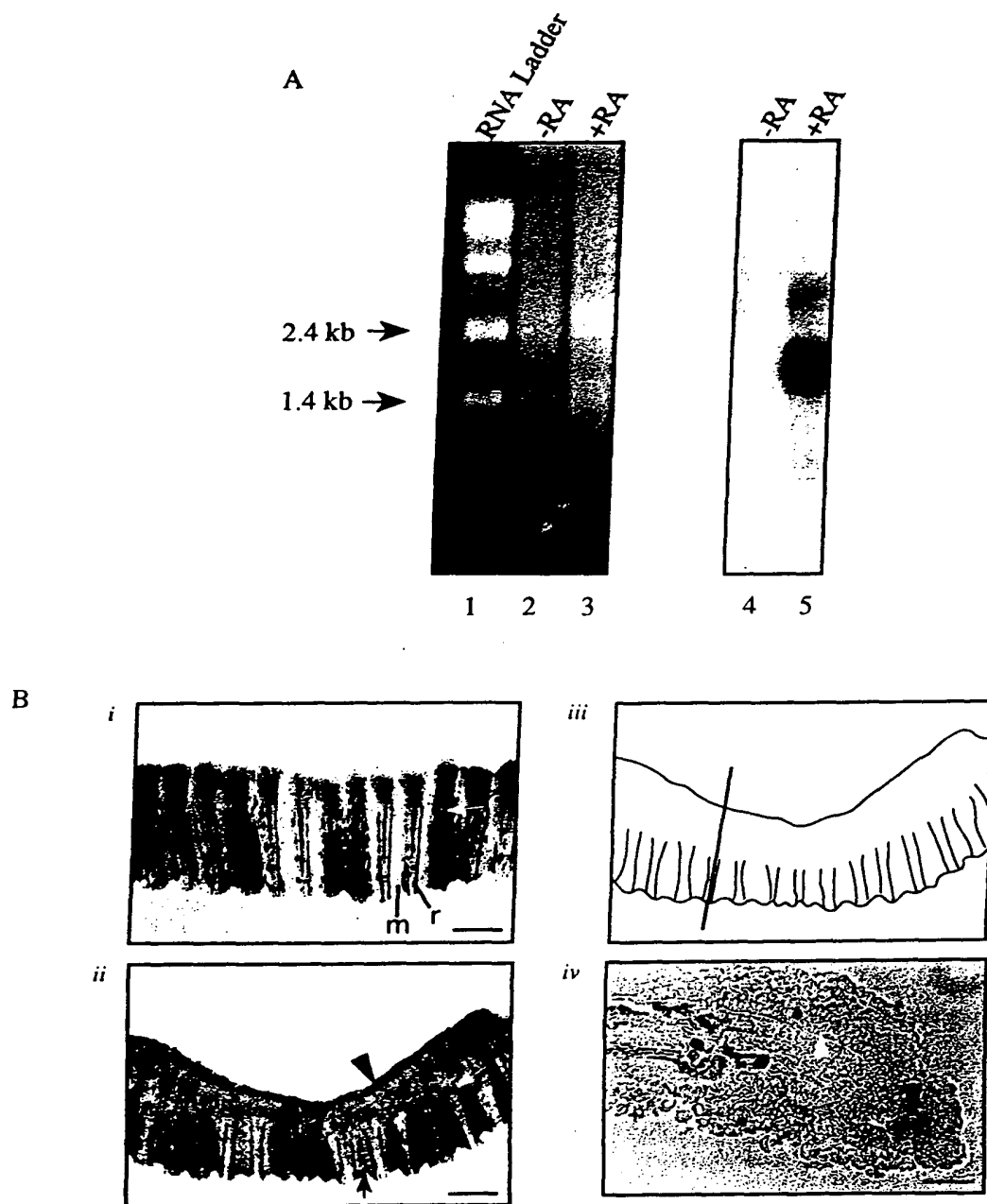
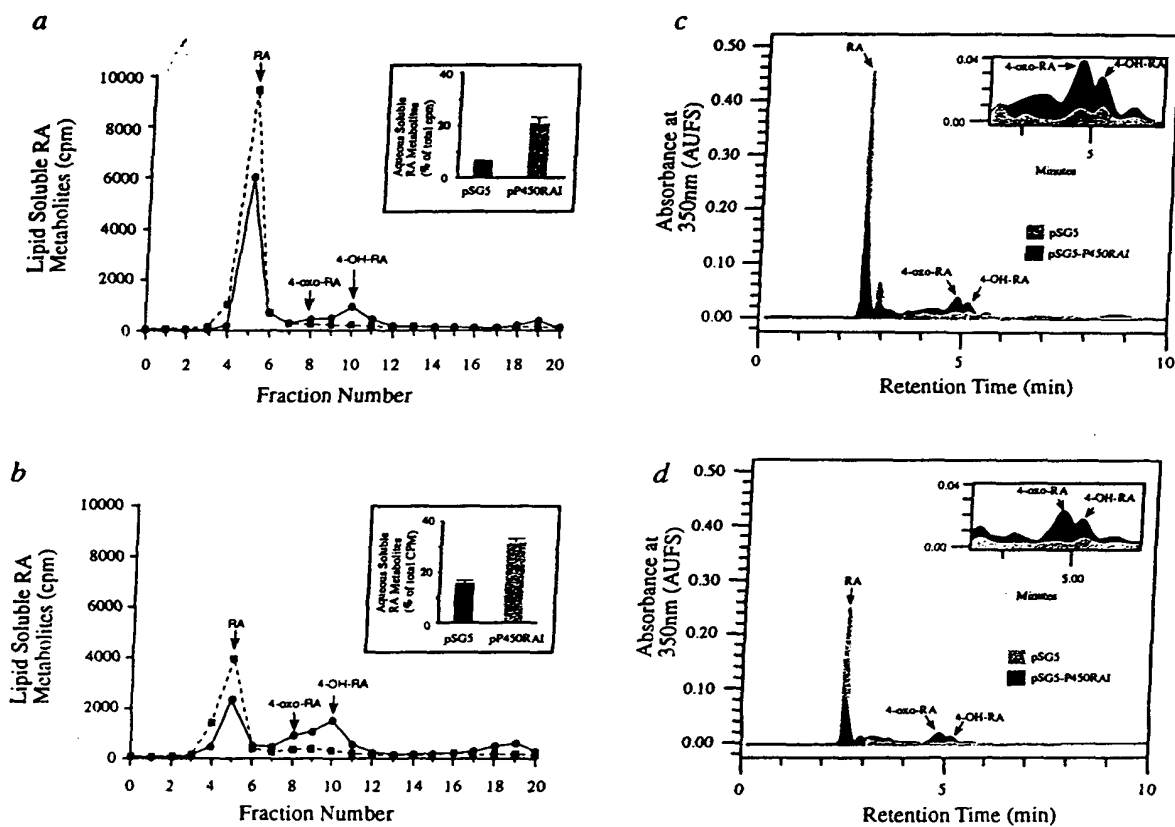


FIG. 3

FIG. 4



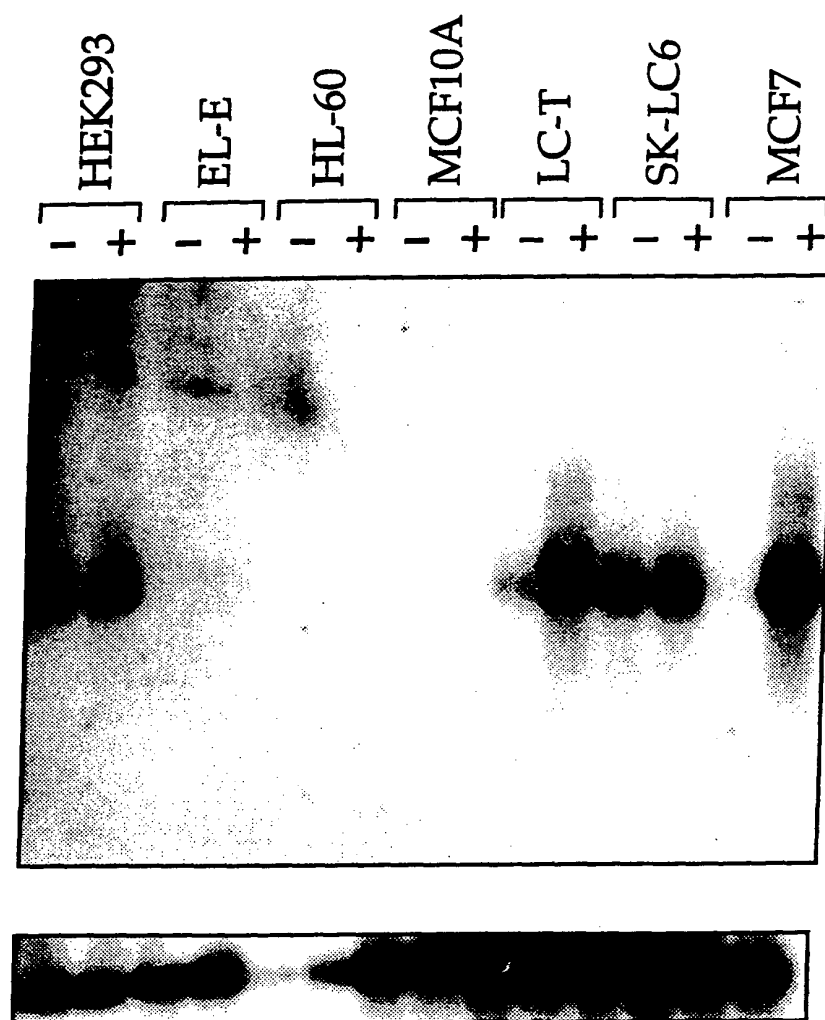


FIG. 5

005260" 28489960

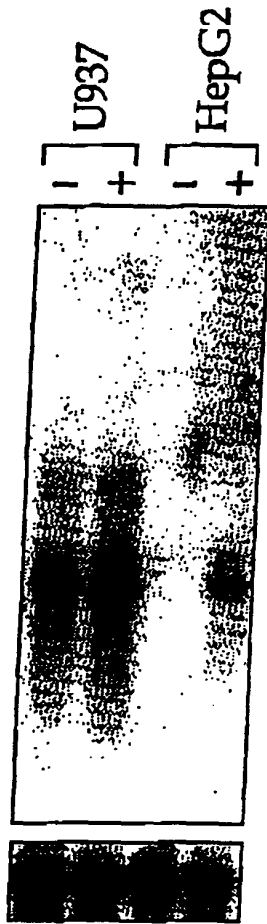


FIG. 6

005260" 28489960

NT2

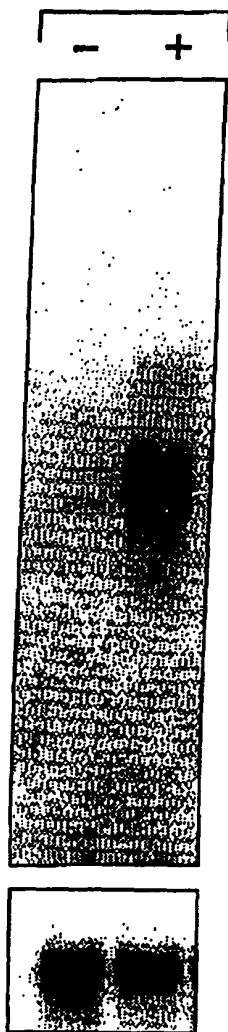


FIG. 7

005260 " 28489960

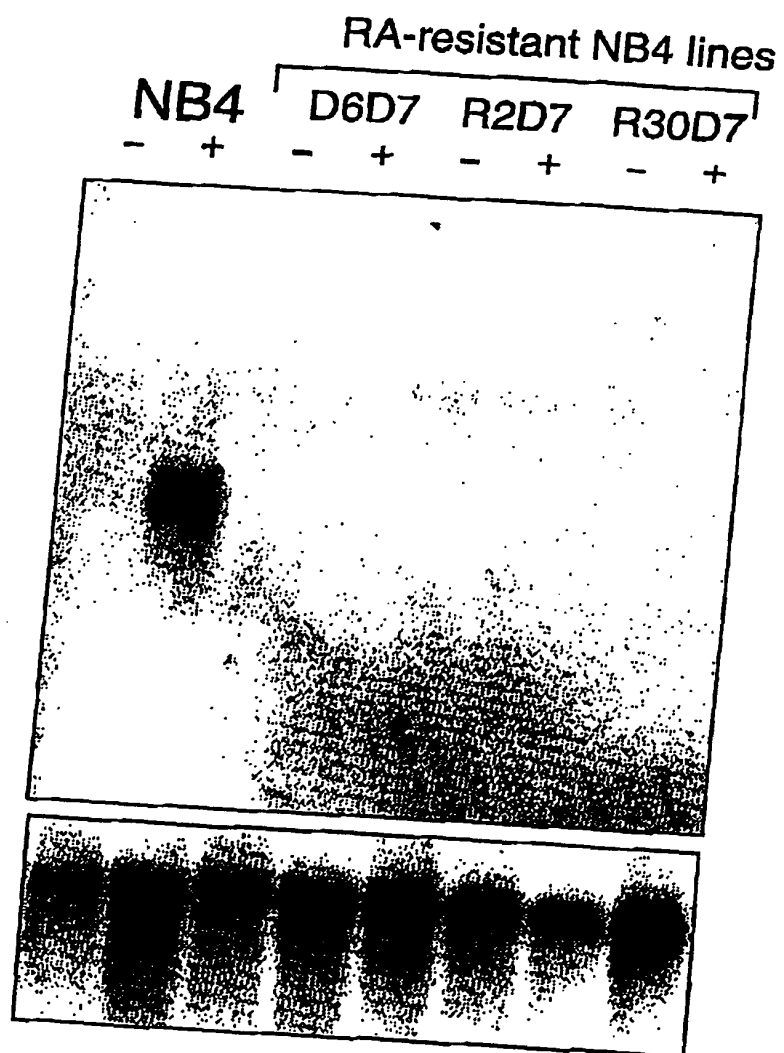


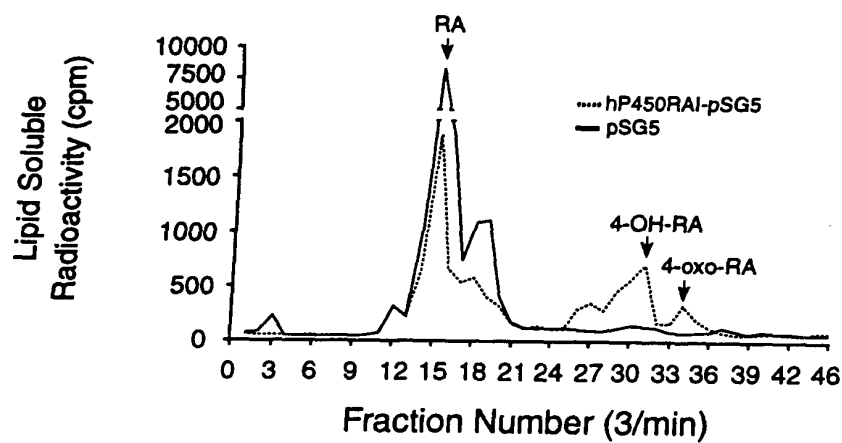
FIG. 8

FIG. 9

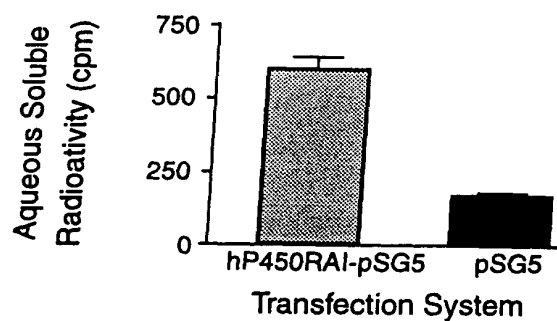
mp450RAI	MGLPALLASALCTFVLPLLLFLAALKLWDLYCVSSRDRSCALPLPPGTMGFPFFGETLQM	60
hp450RAII.....G.....	60
zP450RAI	...YT.MVTF...I...V.....V...EMLMIRRV.PN.RS.....L..I....L	60
mp450RAI	VLQRRKFLQMKRRKYGFYKTHLFGRRPTVRVMGADNVRRILLGEHRLVSVHWPASVRTIL	120
hp450RAIDD.....	120
zP450RAI	I.....R...Q...C.....N.....Q.....K...Q.....	120
mp450RAI	GAGCLSNLHDSSHKQRKKVIMQAFSREALQCYVLVIAEEVSSCLEQWLSCGERGLLVYPE	180
hp450RAI	.S.....R.....E...P..T...G.S.....	180
zP450RAI	.SDT...V.GVQ..NK..A..R....D..EH.IP..QQ..K.AIQE..Q-KDSCV....	179
mp450RAI	VKRLMFRIAMRILLGCEPGPAGGGEDEQQLVEAFEEMTRNLFSLPIDVPFSGLYRGVKAR	240
hp450RAIQL..D.DS.....M...	240
zP450RAI	M.K.....F..EQI--KT...E.....IK.....LR..	237
mp450RAI	NLIHARIEENIRAKIRRLQATEPDGGCKDALQLLIEHSWGERLDMQALKQSSTELLFG	300
hp450RAIQ.....CG.R.S.AGQ.....	300
zP450RAI	.F..SK.....K..QDDDNENEQ-KY.....N.RRSD.PFSL..M.EAA.....	296
mp450RAI	GHETTASAATSLITYLGLYPHVLQKVREEIKSKGLLCKSNQDNKLDMETLEQLKYIGCVI	360
hp450RAIL.....I.....	360
zP450RAIT....VMF...NTE.V.....VQE.VEMGMYTPGKG.S..L.D....T....	356
mp450RAI	KETLRLNPPVPGGFRVALKTFELNGYQIPKGWNVYISICDTHDVADIFTNKEEFNPDRFI	420
hp450RAIE.....S	420
zP450RAII.....V.P.....Q.E..M	416
mp450RAI	VPHPEDASRFSFIPFGGGLRSCVGKEFAKILLKIFTVELARHCDWQLLNGPPTMKTSP TV	480
hp450RAI	A.....	480
zP450RAI	SKGL..G...NY.....S.M.....V....L...TQ..N.I.S.....G..I	476
mp450RAI	YPVDNLPARFTYFQGDI	497
hp450RAIH.H.E.	497
zP450RAITK..SYVRN-	492

005260" 28489960

a



b



c

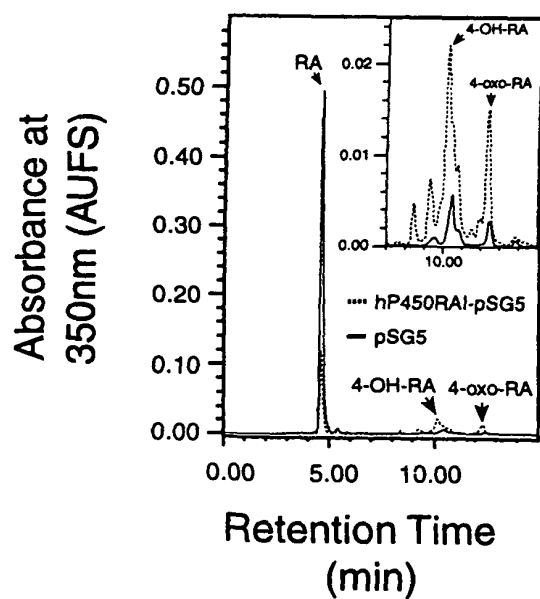


FIG. 10

4-oxo-RA Production in Transfected COS-1 Cells

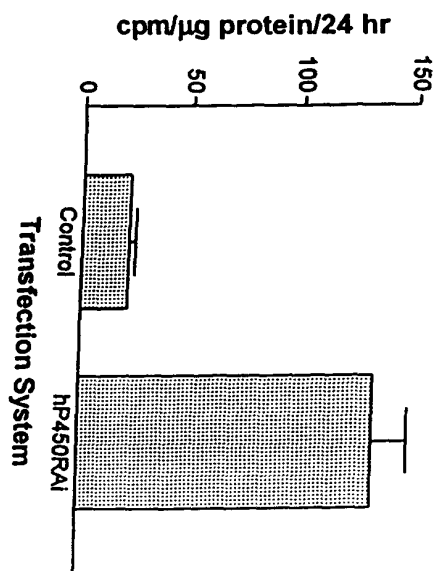


FIG. 11(a)

4-OH-RA Production in Transfected COS-1 cells

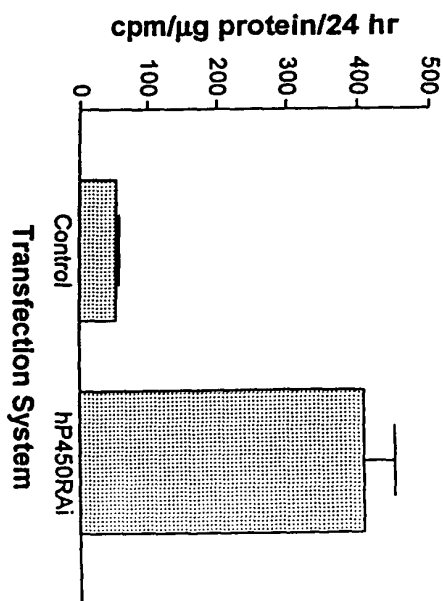


FIG. 11(b)

Formation of Aqueous Soluble Metabolites in Transfected COS-1 Cells

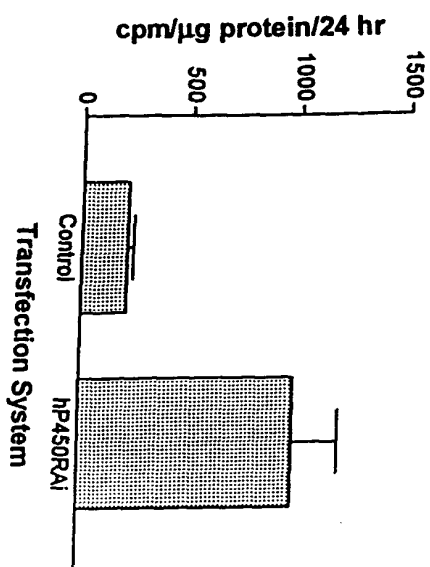


FIG. 11(c)

Unmetabolized RA in Transfected COS-1 Cells

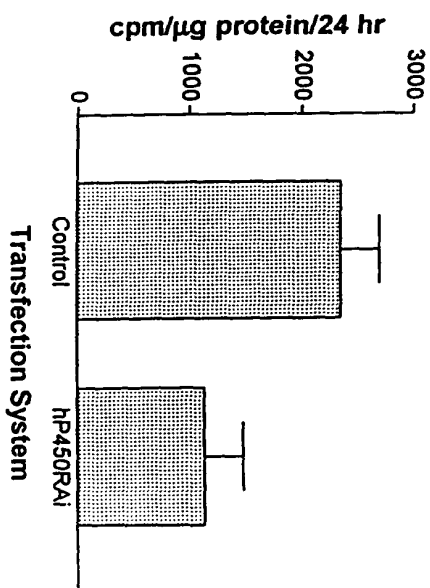


FIG. 11(d)

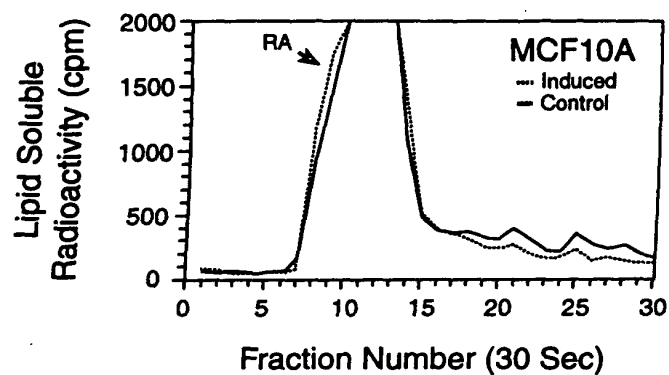
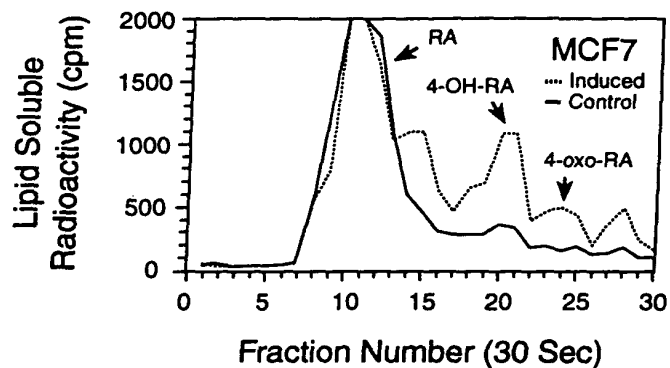
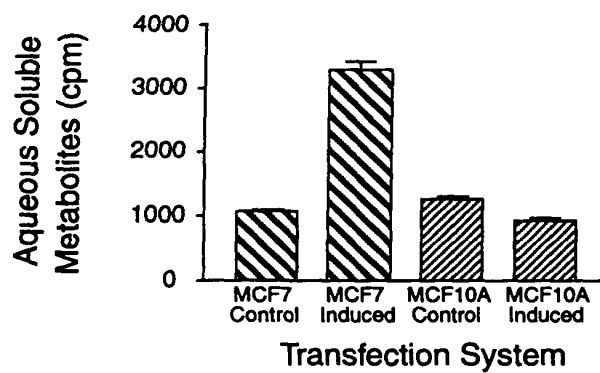
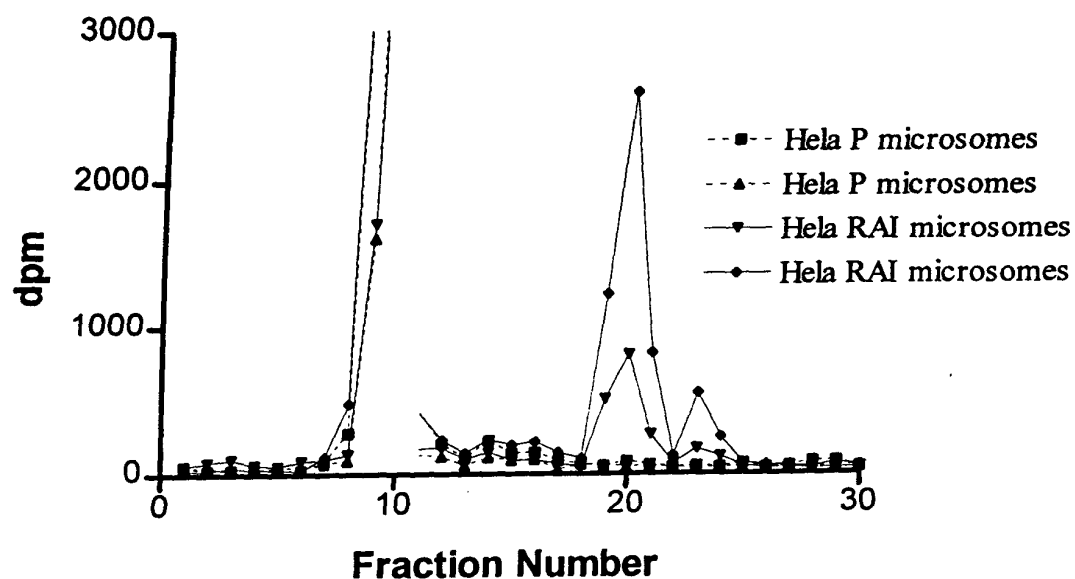
a*b**c*

FIG. 12

005260" 28789960

Microsomal Preparations (90 minutes)



Microsomal Preparations (90 minutes)

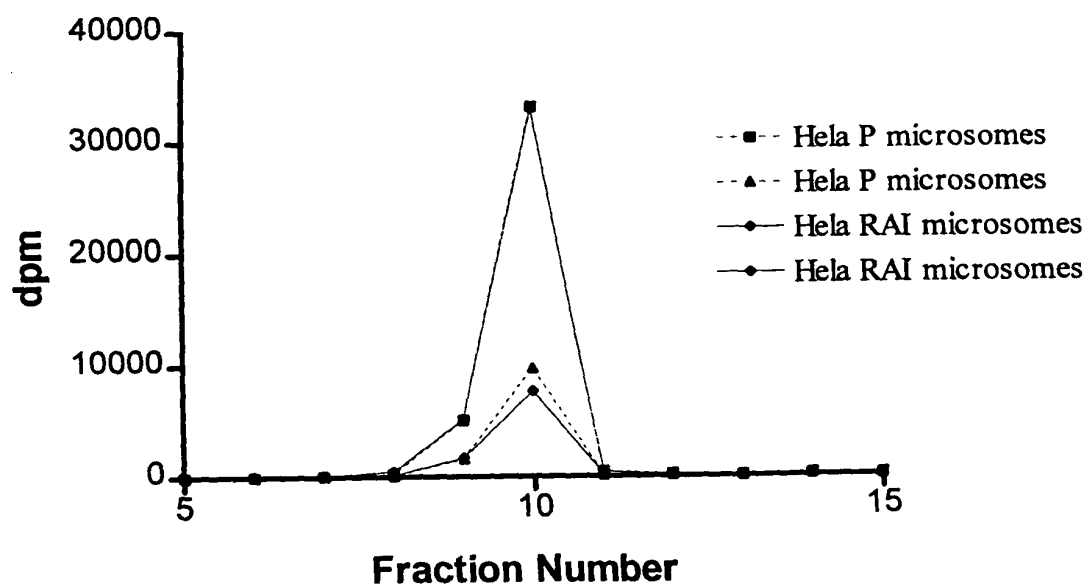


FIG. 13

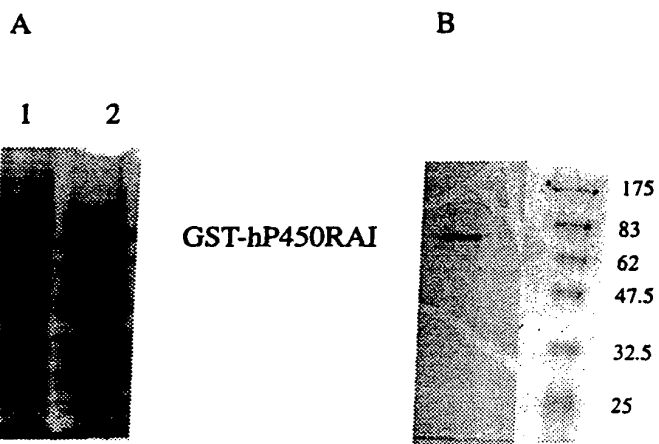


FIG. 14

005260" 20499960

hRAI promoter	-CG-----CA-C-----CC-----CAGGAGG
mRAI promoter	-CG-----CACC-----CC-----CAGGAGG
zRAI promoter	TCGGGGGAATTAAACACCTTTTCAAAGTGAAATCTCAGGATTGTCTGCCTTCTACAGGAGG
hRAI promoter	-----CGCGCTCGGAGGGAA--GCCGCCAC--CGCCGCCCTCTGCCTCGGCG
mRAI promoter	-----CGCGCTCAGAGGGAA--GCCGCCAG--TG-CGCCGCTCTGCCTCGGCG
zRAI promoter	TGGTATTAAATGCGCCTATAACAAATGGTTGAGAGTTTGGAGCCGCTTCTGCCCTG---
hRAI promoter	CGGAACAAACGGTTAAAGATTTT---GGGCCASCGCCTCCGCGGGGGAGGAGCCAGGGG
mRAI promoter	CGGAACAAACGGTTAAAGATTTT---TGGGC-AGCGCCT-CGAGGGGGAGGAGCCAGGGG
zRAI promoter	-----TGGGC-----GGGCGAG-----ATG---
hRAI promoter	CCCCAATCCCGCAATTAAAGATGAACCTTTGGTGAACCTAATTGTCTGACCAAGGTAACG
mRAI promoter	CCCGA--TCCGCAATTAAAGATGAACCTTTGGTGAACCTAATTGTCTGACCAAGGTAACG
zRAI promoter	-----ACACCACAATTAAAGATGAACCTTTGTGAACCTAATTATCTGAGGAAGTTAACA
hRAI promoter	TGGGCAGCAACCTGGGCGGCC---TATAAGCGGCAGCGCCGTGGGGTTTGAAGCGCTG
mRAI promoter	TGGGCAGTAACCTGGGCGGCC---TTATAAGAGGGCGCGCGGGGTTTCGGAGCTAGG
zRAI promoter	GGAGGAGACCTGCGCGCAATGGATATATAAGGCGCGCAGCGAGGACGCCCTCAGTTTG
hRAI promoter	GCGGCGGCGGCAGGTGGCGCGGGAGGTCG-----CG
mRAI promoter	GCGGCGGCGGCAGGTGGCGCGGGAGGCTG-----AA
zRAI promoter	TGCGTAAAGACGCGTCTCCTCTCCAGAAGCTTGTTCGTTTGGCGATCAGTTGCGCG
hRAI promoter	GCGGCGCATGG
mRAI promoter	GCGTGCCATGG
zRAI promoter	CTTCAACATGG

FIG. 15

09668482, 092500

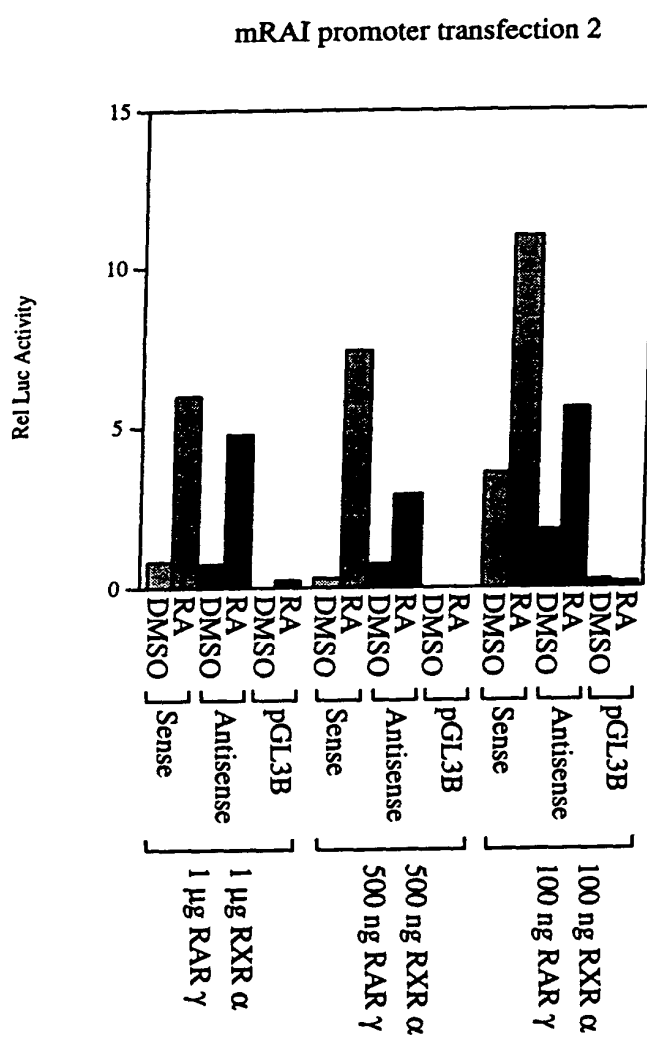
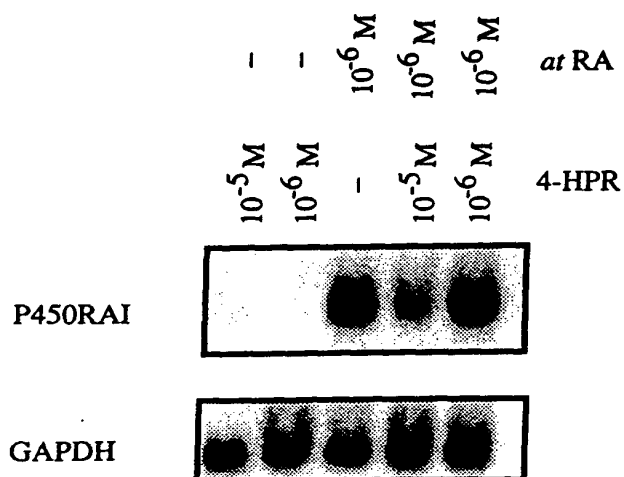


FIG. 16



Cells were treated for twelve hours with the indicated concentrations of *all-trans* retinoic acid (*at*RA) and 4-HPR. Total RNA was extracted using TRIzol, and, following electrophoresis, Northern blotting was performed as described. The nitrocellulose was probed with radiolabelled P450RAI or GAPDH.

FIG. 17

005260" 28789960

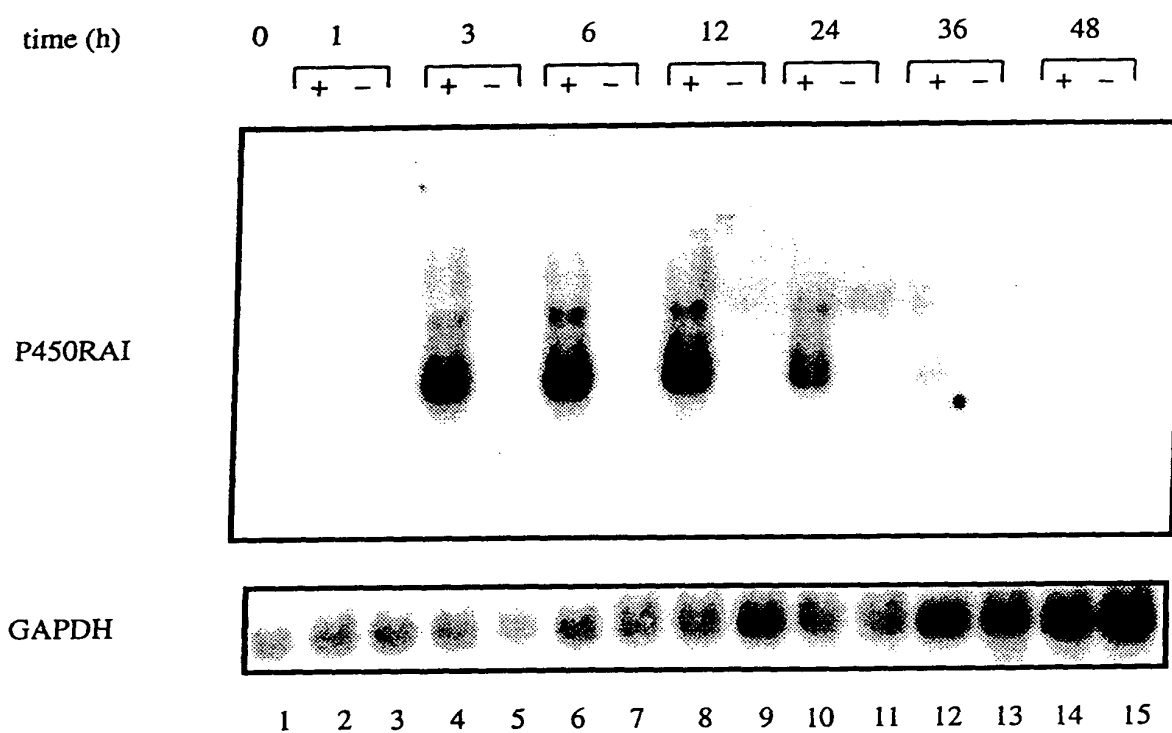


FIG. 18

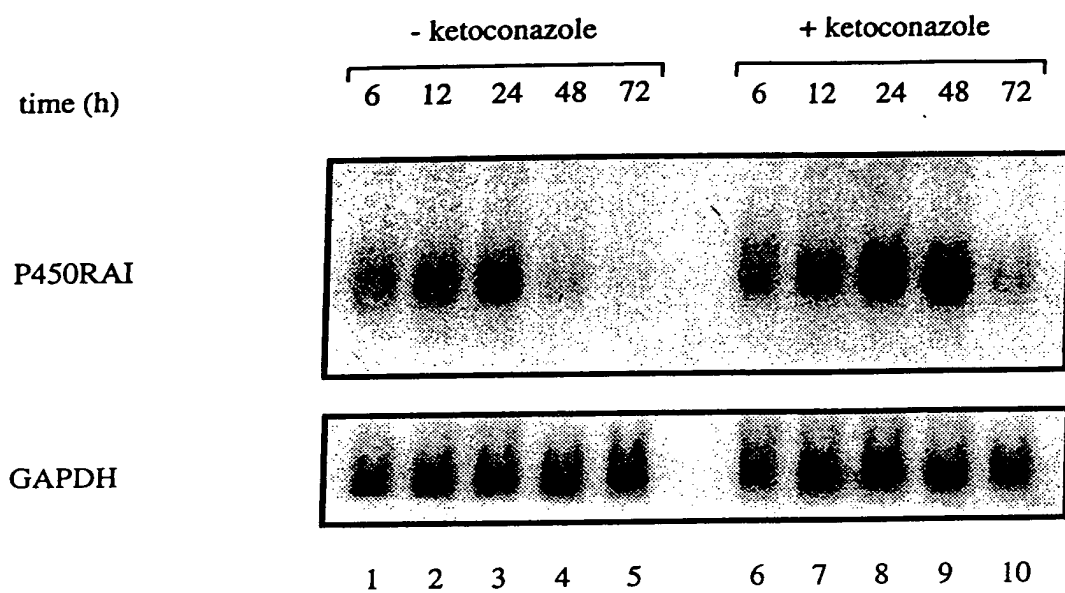


FIG. 19

005260" 28183360

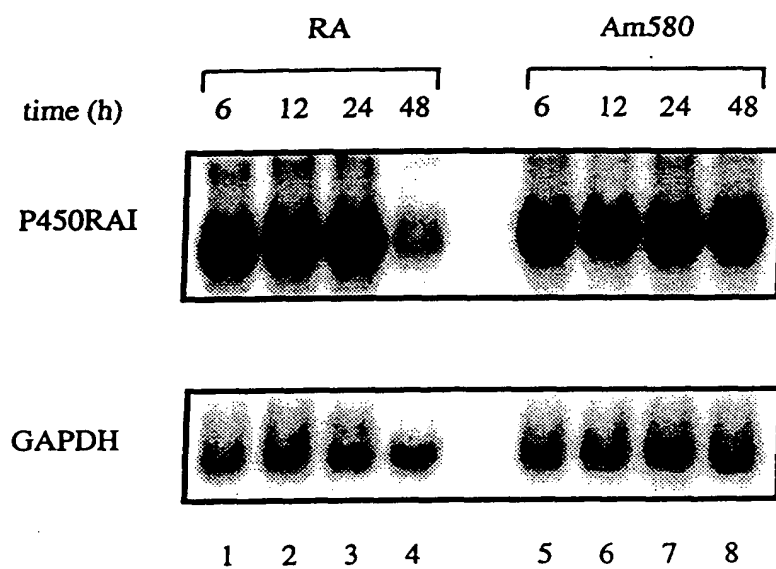


FIG. 20